SEDATIVE, ANALGESIC AND CARDIOPULMONARY EFFECTS OF MIDAZOLAM-BUTORPHANOL PREMEDICATION IN WATER BUFFALOES (BUBALUS BUBALIS)

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ABSTRACT

The study was conducted in 12 water buffaloes of either sex, aged 3 to 8 years and weighing 400-500 kg, to evaluate and compare the sedative, analgesic and cardiopulmonary effects of intravenous midazolam-butorphanol combination with midazolam. Animals were randomly divided into two groups: Group I (midazolam) and group II (midazolam-butorphanol) having six animals in each group. Midazolam (0.2 mg/kg, i.v.) in group I and midazolam-butorphanol (0.2 mg/kg and 0.02 mg/kg, i.v.) combination in group II was used for premedication. Thiopentone sodium (5%) (10 mg/kg, i.v) was used as induction agent in both the groups. Better degree of sedation, analgesia and muscle relaxation was observed in midazolam-butorphanol group. Heart rate decreased significantly after premedication in midazolam-butorphanol group. Respiratory rate decreased non-significantly while rectal temperature decreased significantly (p<0.01) after premedication in both the groups. Halothane concentration required to maintain adequate depth of anaesthesia was lower in midazolam-butorphanol group. The results showed that midazolam-butorphanol combination (0.2 mg/kg and 0.02 mg/kg) can be used safely for premedication during halothane anaesthesia in water buffaloes as this combination provided adequate sedation, analgesia and muscle relaxation with only transient changes in cardiopulmonary parameters.

Keywords: midazolam, butorphanol, halothane, thiopentone sodium, water buffaloes

INTRODUCTION

Use of sedatives before induction of anaesthesia is well established in veterinary practice. Preoperative use of sedatives improve the quality of induction and decrease drug related adverse effects by reducing the amount of injectable and inhalant anaesthetics (Kojima et al., 2002; Sano et al., 2003). Midazolam has mild cardiovascular and respiratory effects and is commonly used as a mild tranquillizer, muscle relaxant and anticonvulsant (Lemke, 2007). It has supra-additive effect with opioids and barbiturates. The combined effect of barbiturate and benzodiazepine is mediated through benzobarbiturate-GABA receptor supramolecular complex in which each site when occupied modulates the other (Tverskoy et al., 1988; Vinik et al., 1994). Butorphanol, an opioid agonist-antagonist has good analgesic, antitussive and

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sedative effect (Pfeffer et al., 1980). It induces only mild sedation and has minimum adverse effects to the cardiovascular system (Greene et al., 1990; Trim, 1983). There are only few reports available on the use of midazolam-butorphanol combination as preanaesthetic in water buffaloes. The present study was therefore, designed to evaluate the sedative, analgesic and cardiopulmonary effects of midazolam-butorphanol combination in water buffaloes.

**MATERIALS AND METHODS**

The study was conducted in 12 water buffaloes of either sex, aged 3-8 years and weighing 400-500kg. Buffaloes were randomly divided into two groups: group I (M) and group II (MB) having 6 animals in each group. All buffaloes were kept off feed for 24 h and water was withheld for 12 h prior to onset of anaesthesia. Different surgical procedures like ventral hernia (n=3), plating (n=3), diaphragmatic hernia (n=3), prepubic tendon rupture repair (n=2), excision of large tumorous mass on neck (n=1) were performed with buffaloes restrained either in lateral or dorsal recumbency. Midazolam (0.2 mg/kg i.v.) in group I (M) and midazolam (0.2 mg/kg, i.v.) + butorphanol (0.02mg/kg, i.v.) combination mixed in a single syringe in group II (MB) was used for premedication. Thiopentone sodium (5%) was used as induction agent in both the groups. Following induction, jaw was opened using a mouth gag and endotracheal intubation was performed. Anaesthesia was maintained with halothane and oxygen mixture via a semi-closed rebreathing system. The vaporizer setting was adjusted according to depth of anaesthesia after monitoring animal’s response to various reflexes. Haemoglobin oxygen saturation value was obtained with the help of pulse oxymeter. Systolic, diastolic and mean arterial pressure was measured with the help of non-invasive blood pressure (NIBP) monitor whose cuff was applied around the base of tail.

The degree of sedation, analgesia and muscle relaxation was graded on 1 to 4 scoring scale. Onset of sedation was recorded by observing behavioural changes after premedication and was graded as: 1 (no sedation) = animal standing alert with its head high and all reflexes present, 2 (mild sedation) = decreased alertness with no reduction in palpebral and pin prick reflexes; 3 (moderate sedation) = animal calm, minimal restraint needed, eyelids partially closed, sluggish palpebral reflex and partial ventromedial rotation of eye; 4 (deep sedation) = animal completely calm, no restraint needed, eyelids closed, very weak palpebral reflex, complete ventromedial rotation of eye.

The quality of induction was evaluated five min after administration of thiopentone sodium and was graded as 1 (poor) = animal excited, frequent attempts to stand after recumbency, massive regurgitation and inability to intubate trachea; 2 (moderate) = mild excitement, mild regurgitation, slightly longer tracheal intubation time and slightly prolonged induction; 3 (good) = no excitement, no regurgitation, no gag reflex, 4 (excellent) = smooth and rapid induction, easy and quick tracheal intubation, no regurgitation. Quality of analgesia was recorded by observing animal’s response to deep pin prick with a 22 G hypodermic needle at every 15 minutes interval. Analgesia was graded as 1 (no analgesia) = strong response to pin prick; 2 (mild analgesia) = weak response to pin prick; 3 (Moderate analgesia) = occasional response to pin prick; 4 (Excellent analgesia) = no response to pin prick.

Extent of muscle relaxation was recorded
by observing relaxation of muscles of limb, jaw and tail and was graded as: 1 (no relaxation) = tightly closed jaws and stiff limbs; 2 (mild relaxation) = moderate resistance to opening of jaw and bending of limbs; 3: moderate relaxation (mild resistance to opening of jaw and bending of limbs; 4 (good relaxation) = no resistance to opening of the jaw and bending of the limbs.

The degree of abolition of palpebral, corneal, pin prick and rectal pinch reflex was graded as: 1 (intact and strong reflex); 2 (mildly depressed reflex); 3 (sluggish reflex); 4 (complete loss of reflex). The extent of salivation was graded as 1 (no salivation); 2 (mild salivation); 3 (moderate salivation); 4 (profuse salivation).

Quality of recovery was graded as 1 (poor) = prolonged struggling, premature attempts to stand; 2 (moderate) = transient excitement along with some struggling; 3 (good) = smooth, easy transition to alertness, resumption of sternal position, 4 (excellent) = smooth, excitement free, animal standing of its own.

Physiological parameters like heart rate (HR) (beats/min), respiratory rate (RR) (breaths/minute) and rectal temperature (RT) (°C) was recorded at base i.e. 0 minute, 5 minutes after premedication and at 5, 15, 30, 45, 60, 90 and 120 minutes after induction of anaesthesia. Haemodynamic parameters like systolic blood pressure (SBP) (mm of Hg), diastolic blood pressure (DBP) (mm of Hg), mean arterial pressure (MAP) (mm of Hg) and haemoglobin oxygen saturation (SpO2) (%) were recorded at the above time interval in both the groups.

**Statistical Analysis**

Analysis of variance (ANOVA) and Duncan’s multiple range test (DMRT) was used to compare the means at different time intervals between the two groups. Paired ‘t’ test was used to compare the means at different intervals with their respective base values in each group. For non-parametric observations the Kruskal-Wallis one way test (Stiegel and Castellan, 1988) was used to compare the means between the two groups.

**RESULTS AND DISCUSSION**

The study was conducted to evaluate and compare the sedative, analgesic and cardiopulmonary effects of midazolam-butorphanol combination with midazolam in water buffaloes. Addition of butorphanol to midazolam brought about clinically appreciable changes in sedation, analgesia and muscle relaxation without any adverse effects on cardiopulmonary parameters. The quality of sedation was better in group II (MB) as compared to group I (M) and the difference between two groups was statistically significant (p<0.05). Mean sedation scores of 2.75±0.40 and 3.75±0.25 were reported in group I (M) and II (MB), respectively. In group I, out of six buffaloes, two gained score 4, three scored 3 and remaining one scored 2 while in group II(MB), out of six buffaloes, four gained score of 4 and remaining two scored 3 (Figure 1). Signs of sedation appeared early in buffaloes of group II (MB) (30 sec to 1 minute) as compared to group I (M) (1 to 2 minutes). Excitement immediately after premedication was observed in 2 buffaloes belonging to group I (M) while none of the buffaloes in group II (MB) showed any signs of excitement. Better sedation produced by midazolam (0.2 mg/kg b.wt.) + butorphanol (0.02 mg/kg b.wt.) combination in group II (MB) might be due to combined sedative effect of both the drugs. Midazolam as a single agent has a mild sedative effect but it shows additive or synergistic
activity when administered with other sedatives (Cwiek et al., 2009). The analgesic and sedative effect of butorphanol is due to two subtypes of μ receptors: μ1-receptors that act above the level of the spinal cord, and μ2-receptors that act within the spinal cord (Boothe, 2001).

Moderate quality of induction was observed in buffaloes of group I (M), with a mean induction score of 3.25 ± 0.25. In group II (MB), the quality of induction was good, with mean induction score of 3.75 ± 0.25 (Figure 2). Laryngeal reflex diminished early in group II (MB) and the time taken for endotracheal intubation was significantly (p<0.05) less in group II (MB) (26.25 ± 1.75) as compared to group I (M) (30.00 ± 3.39). A synergistic interaction between midazolam and butorphanol could be responsible for the abolition of laryngeal reflex and better conditions for endotracheal intubation in group II (MB). Similar synergism between opioid and midazolam was observed in humans (Ben Shlomo et al., 1990) and animals (Kissin et al., 1986).

Significant (p<0.05) increase in the score for palpebral reflex was observed after premedication in group II (MB) while in group I (M), the increase was non-significant (Figure 4). A non-significant increase in the score for corneal reflex followed by a significant (p<0.05) increase in the score for rotation of eyeball was observed within 5 minutes of preanaesthetic administration in both the groups. Moderate abolition of palpebral and corneal reflex observed after premedication in both groups was found similar to the observations of Kaur and Singh (2004) who reported loss of eyelash reflex, mild to moderate palpebral reflex and full corneal reflex after midazolam administration (0.2 mg/kg i.v.) in bovines. Completely ventral and ventromedial rotation of eyeball for a longer duration was observed in buffaloes of group II (MB) as compared to group I (M). Analgesia with complete abolition rectal pinch reflex for longer duration was observed in buffaloes of group II (MB) as compared to group I (M) (Figure 5). Midazolam does not have analgesic property, however addition of μ opioid agonist butorphanol in group II (MB) might have resulted in deeper and adequate level of analgesia (Pfeffer et al., 1980). Midazolam is found to have a considerable effect on the nociceptive transmission in superficial dorsal horn (Kohno et al., 2006) and cause pain relief (Akhhlaghi and Rajaee, 2008). Similar findings were reported by Itamoto et al. (2000) in dogs, where addition of 0.1 mg/kg butorphanol to medetomidine or midazolam produced adequate analgesic effect.

The extent of muscle relaxation was assessed by relaxation of jaw tone and limbs, respectively. The score for limb relaxation increased significantly (p<0.05) in group II(MB) compared to a non-significant (p>0.05) increase in group I(M) (Figure 6). Better degree of muscle relaxation observed in buffaloes of group II (MB) might be attributed to the synergistic interaction between midazolam and butorphanol. Midazolam is a benzodiazepine derivative known to have good muscle relaxant action (Hellyer et al., 1991; Ilkew et al., 1998). Although, opioids by themselves do not induce muscle relaxation, however, their additive or synergistic interaction with benzodiazepine might have caused enhanced muscle relaxation in buffaloes of group II (MB).

No statistically significant (p>0.05) difference between the two groups regarding degree of salivation was reported. However, salivation was moderate following midazolam administration in group I (M) while it was mild after midazolam-butorphanol premedication in group II (MB). Court and Greenbalt (1992) and Butola and Singh (2007) reported similar findings in dogs where drooling
of saliva was seen after midazolam administration. Opioids, on the other hand decrease the production of saliva in mouth and this may explain for mild salivation reported after midazolam butorphanol premedication in group II (MB).

Significant (p<0.01) difference in the amount of halothane used was observed between the two groups. Halothane concentration required to maintain adequate depth of anaesthesia was 3.25 ±0.50 % and 2.75 ±0.25 % in group I(M) and group II (MB), respectively (Figure 7). More halothane sparing effect of midazolam butorphanol combination in group II (MB) might be due combined analgesic and/or sedative effects of both the drugs. Midazolam decreases the MAC of potent inhaled anesthetics in humans (Inagaki et al., 1993) and animals (Hall et al., 1988). Studies in humans suggested that midazolam produced marked reduction of halothane MAC at serum concentration lower than that required to cause sleep (Inagaki et al., 1993). A plasma midazolam concentration of 539 ng mL⁻¹ reduced halothane MAC by up to 70% in the same study.

Heart rate decreased significantly (p<0.05) after midazolam-butorphanol administration in group II (MB) while in group I (M), the decrease in heart rate was non-significant (Figure 8). Midazolam has minimal cardiovascular depressant effects (Gross et al., 1990; Tranquilli et al., 1991) and although butorphanol posses less cardiovascular effect than classical opiate agonists, it can cause a decrease in cardiac rate secondary to increased parasympathetic tone and mild decrease in arterial blood pressure (Taylor et al., 1988). Decrease in heart rate after thiopentone administration in both groups of the present study supported the findings in buffaloes administered with thiopentone sodium and glyceryl guiacolate (Agrawal et al., 1983). Significant (p<0.05) decrease in heart rate observed during maintainence period in both groups may be attributed to the hypotensive effect of halothane. Similar observation was reported in cattle (Short et al., 1968) and buffaloes (Gahlawat et al., 1986). No significant difference in respiratory rate was observed after premedication in both the groups. Significant (p<0.01) hypoxemia observed after induction in both the groups might be due to the respiratory depressant effect of thiopentone sodium, as the barbiturates can cause significant cardiovascular and respiratory depression (Carpenter et al., 2005).

Respiratory rate decreased non-significantly after premedication followed by a significant (p<0.01) decrease after induction in buffaloes of both the groups (Figure 9).

Rectal temperature decreased significantly (p<0.05) after midazolam butorphanol premedication in group II (MB) while in group I (M), the decrease was non-significant (Figure 10). Reduced muscle activity along with deep sedation induced by midazolam-butorphanol combination in group II (MB) might have led to the decrease in rectal temperature in this group. Significant (p<0.05) hypothermia observed after induction and throughout maintainence period in both the groups could be due to reduced basal metabolic rate and muscle activity on one hand and depression of thermoregulation on the other which might have resulted in hypothermia (Ponder and Clarke, 1980).

A non significant decrease in blood pressure was recorded after premedication in both the groups while after induction and during maintainence, blood pressure was found to decrease significantly (p<0.05) at few intervals in both the groups (Figure 11). However, in both the groups hypotension was a consistent finding during maintainence with halothane and the severity
of hypotension was closely related to the depth of anaesthesia. Similar hypotensive effect of halothane has also been demonstrated in cattle (Short et al., 1968), dogs (Steffey et al., 1975) and horses (Smith, 1969). Significant (p<0.05) decrease in SpO₂ after premedication in group II (MB) might be due to the respiratory depression caused by the combined effect of sedatives used. A decrease in SpO₂ value after medetomidine and butorphanol anaesthesia was noticed in buffalo calves (Malik, 2008; Ahmed, 2009). Decrease in SpO₂ at few intervals was observed after induction in both the groups (Figure 12). However, at other intervals, decrease in SpO₂ was only transient and was fairly maintained throughout most of the observation period.

The scores for recovery quality in buffaloes of group I (M) and group (MB) were 3.75±0.25 and 3.25±0.25, respectively. No statistically significant difference in the time of recovery was observed between the two groups.

The results showed that the combination of midazolam with butorphanol (0.2 mg/kg and 0.02 mg/kg) for the purpose of premedication in water buffaloes induces high-quality sedation, analgesia and muscle relaxation. The combination considerably reduces the amount of inhalant anaesthetics used and produces transient changes in cardiopulmonary parameters. Midazolam-butorphanol combination can be used safely for premedication during halothane anaesthesia in water buffaloes.

**AKNOWLEDGEMENTS**

The authors are grateful to Dr. S. K. Uppal, Professor, Department of Veterinary Medicene and Dr. S. Prabhakar, Professor-cum-head, Department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab), India for providing the facilities of Diagonostic Clinical Complex. We would also like to thank Dr. Ravi, Senior Scientist, Department of Biotechnology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India and Dr. Samita, Associate Professor, Department of Biostatistics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab), India for their great help in statistical analysis.

**Declaration**

A prospective, randomized, blinded study protocol was used and was approved by the State Veterinary Authorities and informed consent was obtained from the owners. The experiments performed comply with the current laws of the country.

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Figure 1. Mean ± SE of score for sedation quality in group I (M) and group II (MB).

Figure 2. Mean ± SE of score for induction quality in group I (M) and group II (MB).

Figure 3. Mean ± SE of score for Intubation time in group I (M) and group II (MB).
Figure 4. Mean ± SE of score for palpebral reflex in group I (M) and group II (MB).

Figure 5. Mean ± SE of score for rectal pinch reflex in group I (M) and group II (MB).

Figure 6. Mean ± SE of score for limb relaxation in group I (M) and group II (MB).
Figure 7. Mean ± S.E. of halothane (%) used at various intervals group I (M) and group II (MB).

Figure 8. Mean ± SE of heart rate (beats/min) at different time intervals in group I (M) and group II (MB).

Figure 9. Mean ± SE of respiratory rate (breaths/min) in group I (M) and group II (MB).
Figure 10. Mean ± SE of rectal temperature (°C) in group I (M) and group II (MB).

Figure 11. Mean ± SE of mean arterial pressure (mm Hg) in group I (M) and group II (MB).

Figure 12. Mean ± SE of hemoglobin oxygen saturation (mm Hg) in group I (M) and group II (MB).


